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Isoëtes tennesseensis (Isoëtaceae), an Octoploid Quillwort from Tennessee

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ABSTRACT.—*Isoëtes tennesseensis*, an octoploid species with a chromosome count of $2n = 88$, is described. It occurs in the Hiwassee River in Tennessee. Past collections of this species have been misidentified as *Isoëtes macrospora* (= *I. lacustris*). *Isoëtes tennesseensis* differs from *I. lacustris* in chromosome number, megaspore and microspore morphology and distribution. Speculation on the origin of this new species is presented.

In July 1978, Eugene Wofford and Michael Dennis collected quillworts from the Little Tennessee River at Jones Ferry, Tomatlo Ford, the southwestern end of Davis Island, and from the Hiwassee River approximately 1.1 miles southeast of the bridge on highway 411 in Tennessee. These collections, as well as subsequent ones from the Hiwassee River, have been identified as *Isoëtes macrospora* Dur. (Dennis *et al.*, 1979; Boom, 1979; Taylor *et al.*, 1993). The population of *I. lacustris* L. (= *I. macrospora*) in eastern Tennessee is roughly 450 miles from the nearest known outlying population at Passage Creek in northern Virginia (Svenson and Griscom, 1935). Both of these populations are disjunct from the more northern *I. lacustris* (Taylor *et al.*, 1993). Dennis *et al.* (1979) hypothesized that these outlying populations of *I. macrospora* could be the result of either long-range dispersal by waterfowl from northern populations or relics of a previously wider distribution.

Except for the difference in geography, *I. macrospora* and *I. lacustris* are indistinguishable from each other. Chromosome number, as well as leaf and spore morphology is the same. Therefore, *Isoëtes macrospora* has recently been placed in synonymy with the European *I. lacustris* (Taylor *et al.*, 1993).

In North America, *I. lacustris* ranges from Greenland and Newfoundland west to Saskatchewan. It typically occurs in cool, oligotrophic lakes, ponds, and streams. *Isoëtes lacustris* is distinguished by its dark green, rigid leaves and large megaspores that range from 550 to 750 μm in diameter (Taylor *et al.*, 1993). Megaspores typically have a cristate to reticulate ornamentation (Fig. 1 A–C) and a densely papillate girdle below the equatorial ridge (Fig. 1 C). Kott and Britton (1980), Taylor and Luebke (1988), and Britton and Goltz (1991) have reported chromosome counts of $2n = 110$ for *I. lacustris* (Fig. 2 A).

Recent studies of plants from the Tennessee populations have shown that past identifications of these plants as *I. lacustris* are incorrect. These populations represent an undescribed species. In this paper we present our evidence from morphological and cytological studies and describe and name this new taxon.

Materials and Methods

Mature megaspores and microspores were taken from live plants and herbarium specimens. Photomicrographs of spores were obtained with a Hitachi S-570 scanning electron microscope. Measurements of megaspore diameters and microspore lengths were made using Olympus SZX12 and Nikon Microphot-FX microscopes outfitted with ocular micrometers. A minimum of 20 megaspores and 20 microspores were measured from fertile specimens. Megaspores were measured dry while microspores were placed in a drop of water on a slide and covered with a coverslip before being measured.

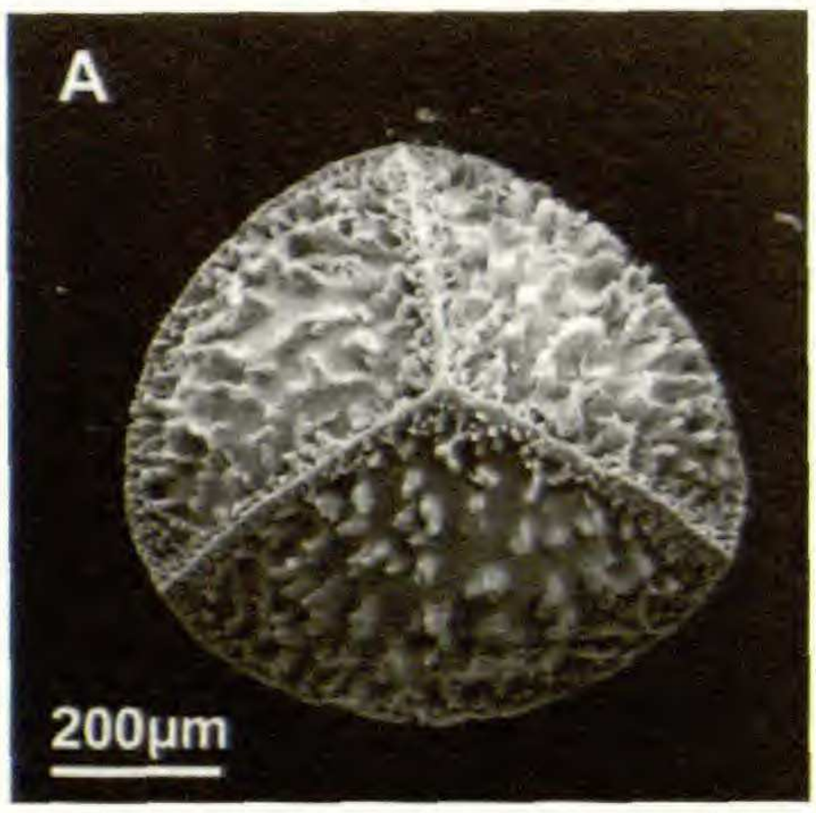
Procedures used for obtaining chromosome counts follow Jong (1997) with some modifications. Plants of *I. tennesseensis* were floated in deionized water in a growth chamber under a cycle of 12 hours of light, 12 hours of darkness and a constant 18 °C until new roots had formed. New roots approximately 6 mm long were harvested in late morning and pretreated in a saturated solution of paradichlorobenzene (PDB) in the dark at room temperature for four hours. Roots were then fixed in Farmer's Solution (3:1 96% ethyl alcohol : glacial acetic acid), left at room temperature for one hour and then stored in the freezer. For staining, roots were hydrolyzed in 1N HCL for ten minutes at 60°C, soaked in three different changes of 96% ethyl alcohol for fifteen minutes each, blotted, placed in Whitman's hematoxylin stain for approximately one hour, and then destained in glacial acetic acid for five minutes.

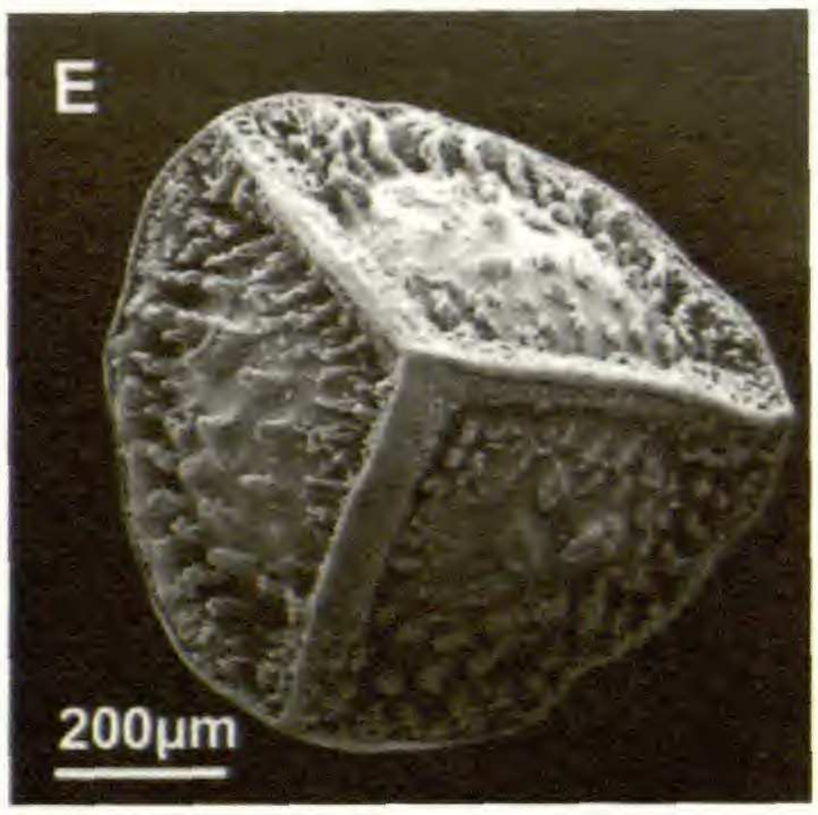
Results

Megaspores of *I. tennesseensis* have bold, broad tri-radiate and equatorial ridges and an obscure to slightly papillate girdle below the equatorial ridge. Ornamentation on the proximal half may be sparse to dense and varies from cristate to rugate (Fig. 1 E–G). The muri on the distal face are bold with even crests, forming a broken to somewhat regular pattern with areolae of various shapes. Megaspore size ranges from 616–946 μm in diameter with a mean of 753 μm in diameter (N = 40; SD = 71.76). These megaspores differ from those of *I. lacustris* in both size and ornamentation. Megaspores of *I. lacustris* are slightly smaller ranging in size from 550–750 μm in diameter (Taylor *et al.*, 1993). Kott and Britton (1983) report a mean diameter of 640 μm. In addition, *I. lacustris* megaspores have a narrow tri-radiate and equatorial ridge, with a densely papillate to occasionally smooth girdle. The ornamentation pattern on the proximal hemisphere is broken, short cristate whereas on the distal face it varies from cristate to nearly reticulate with narrow muri and uneven crests (Fig. 1 A–C).

Microspores of *I. tennesseensis* have a laevigate surface and range in size from 33–40 μm long with a mean length of 36 μm (N = 40; SD = 2.10) (Fig. 1 H). The microspores in *I. lacustris* (Fig. 1 D) are larger in size ranging from 37–50 μm long with a mean of 43 μm long (N = 20; SD = 3.25) and have papillose ornamentation (Kott and Britton, 1983; Taylor *et al.*, 1993).

Chromosome counts from the squashed root tips of eight plants showed that


A
200µm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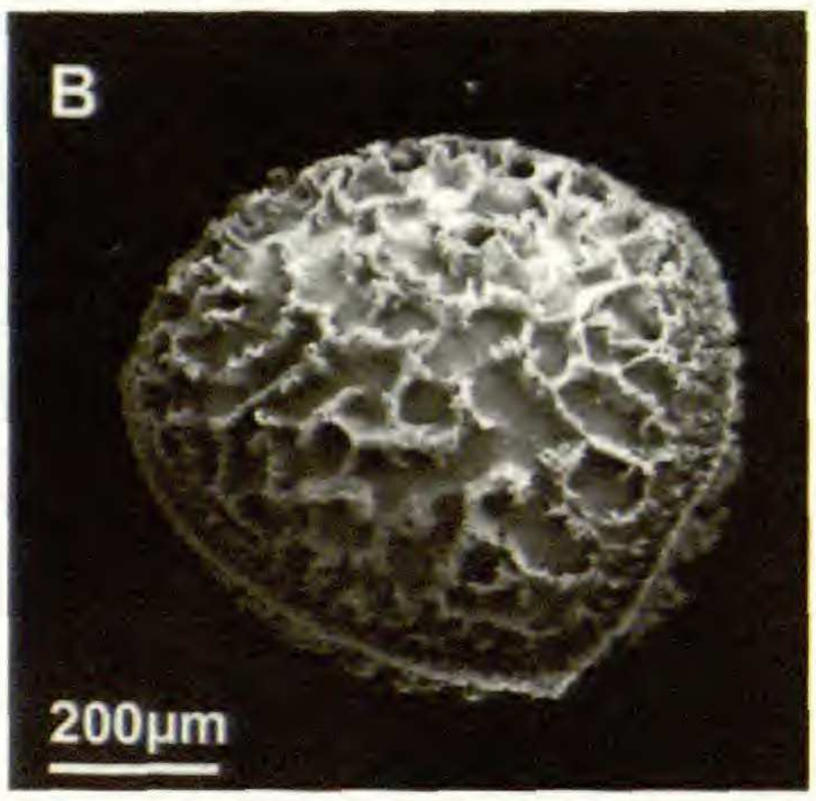
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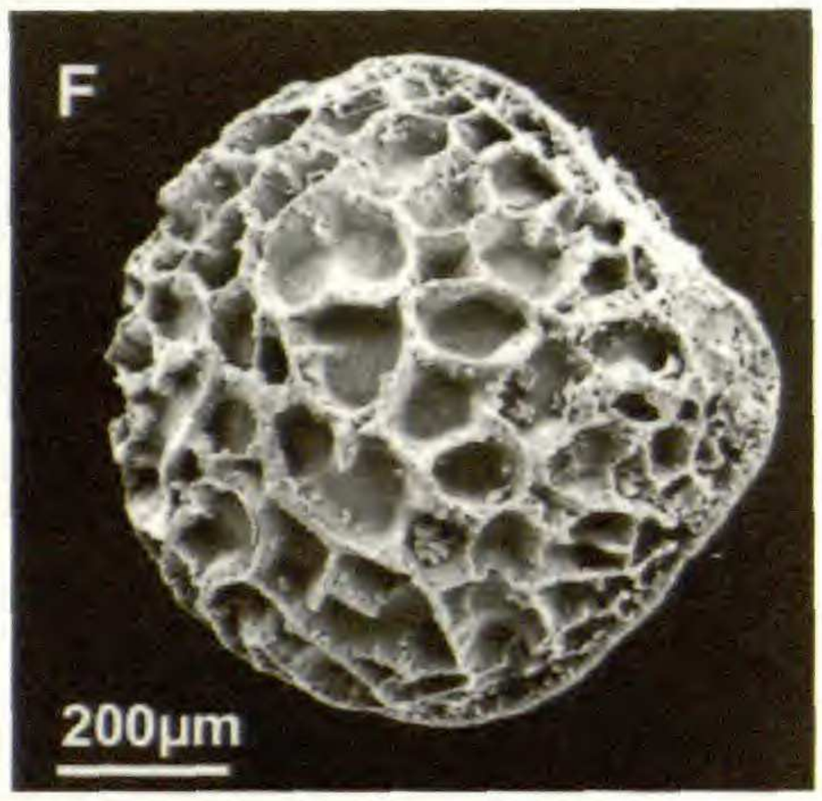
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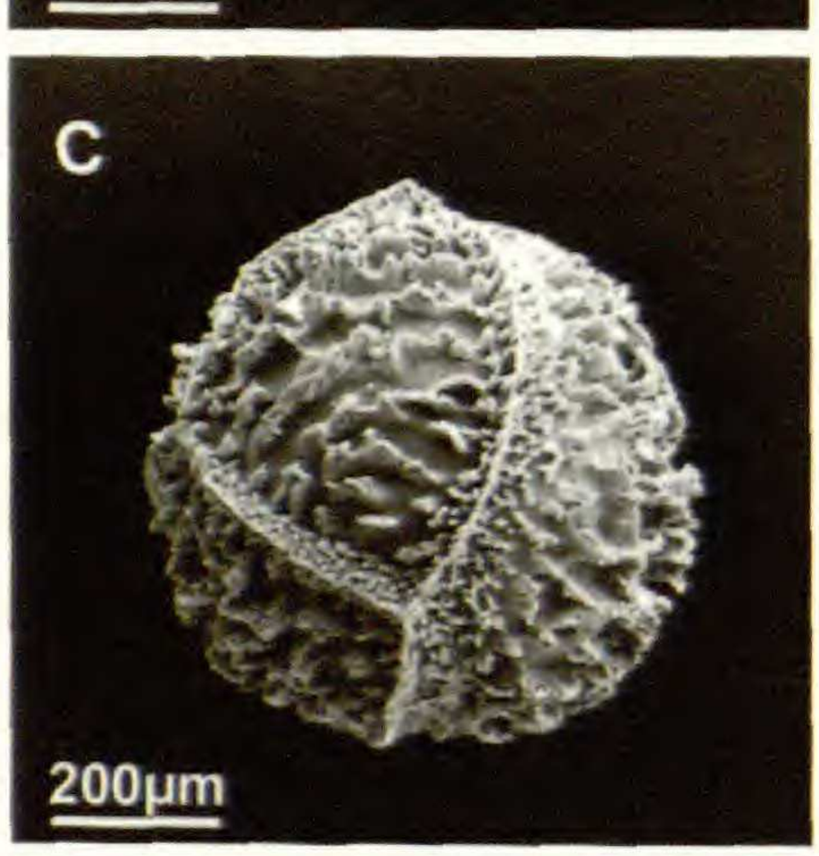
C
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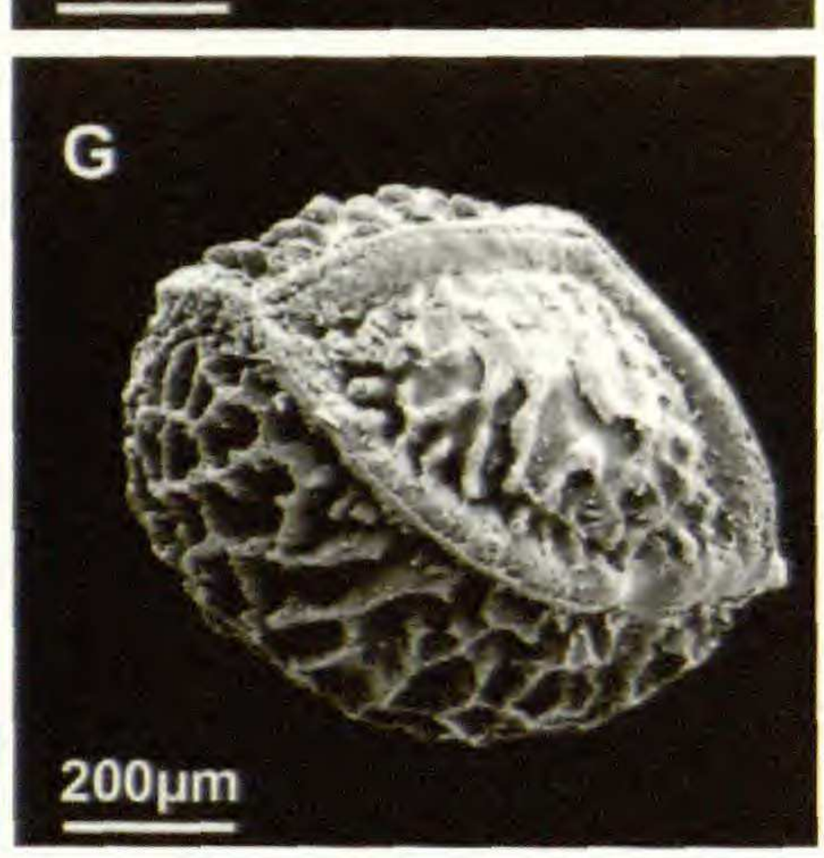
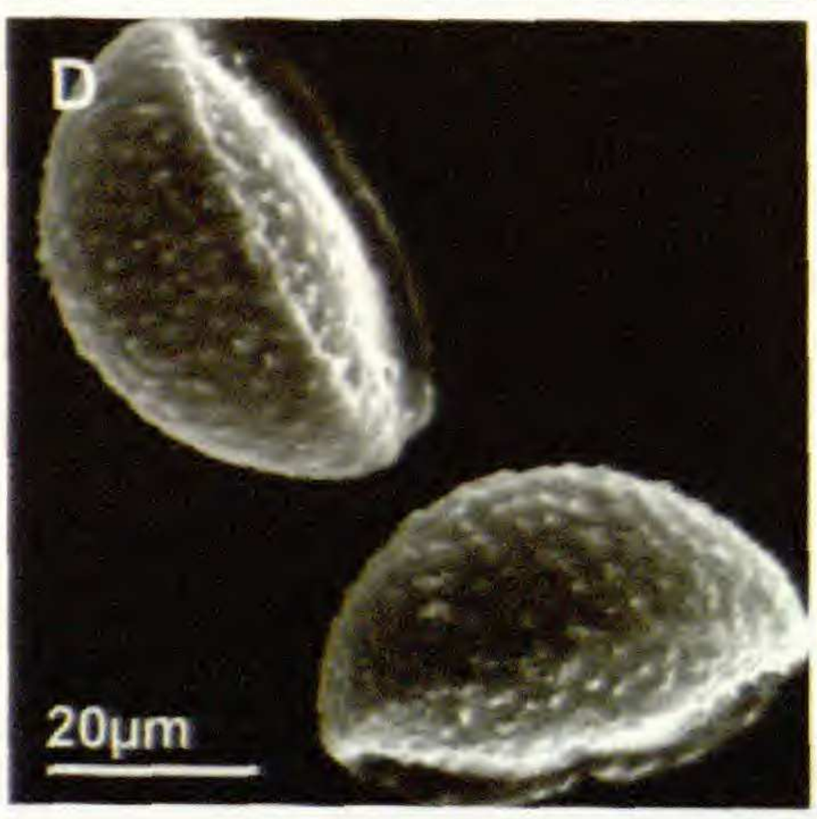
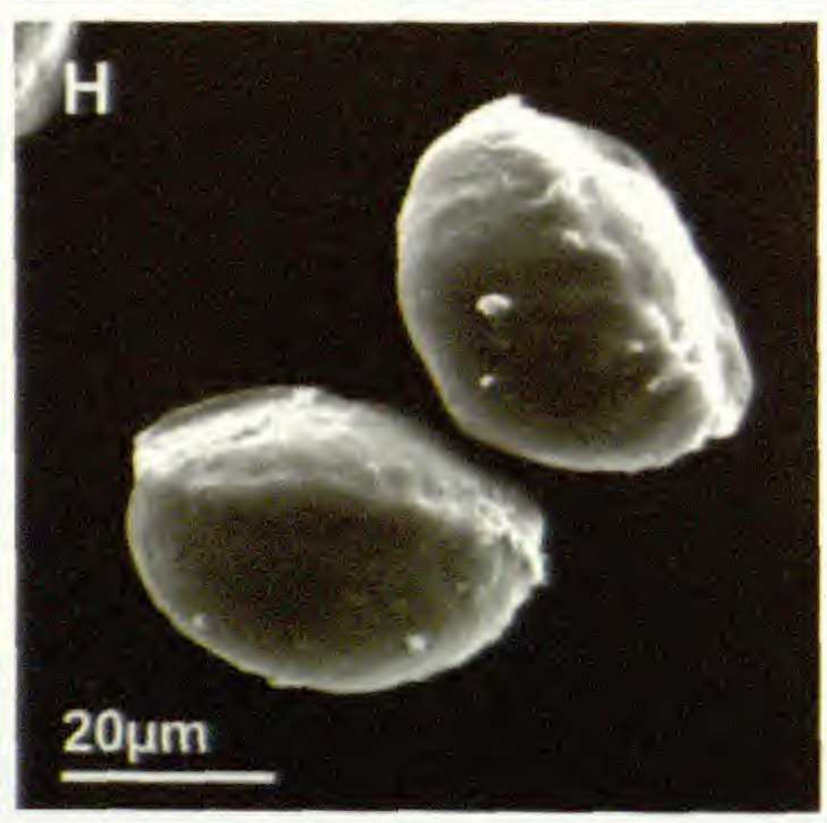

G
200µm


D
20µm


H
20µm

I. tennesseensis is an octoploid, $2n = 88$ (Fig. 2 B), not the $2n = 110$ characteristic of *I. lacustris*. This is the first octoploid species of *Isoëtes* reported for North America.

DISCUSSION

Based on our examination of recent and past collections from the Little Tennessee and Hiwassee Rivers we describe the following new species:

Isoëtes tennesseensis N. T. Luebke & J. M. Budke, ***sp. nov.*** TYPE.—U.S.A. Tennessee: Polk Co., Hiwassee River, ca. 1 mile downstream of the crossing of Tellico-Reliance Road, 15 July 2001, *J. Budke, K. Heafner, E. Lickey and K. Gustafson 17* (holotype: MIL; isotype: MU). **Figs. 1 E–H, 2 B, C.**

Planta aquatica. Caudex bilobatus. Folia 15–35, atro-olivacea, usque ad 11 cm alta, rigida; subula recta usque recurvata apicem versus; alae basim versus, pallida brunneae. Ligula anguste elongata usque triangulata. Labium spathulatum. Velum tegens sporangium <20%. Sporangium basale, ovale, cum maculis brunneis. Megasporae albidae, 616–946 μm diametro, cristato-reticulatae, cum cristis triradiatis et crista aequatoria lata. Microsporae pallide canae in massa, 33–40 μm longae, laevigatae. Chromosomatum numerus $2n = 88$.

Plant aquatic. Rootstock 2-lobed. Leaves 15–35, dark olive-green, up to 11 cm tall, rigid. Subula straight to recurved toward tip, terete in cross-section, ca. 1.5 mm wide at mid length (Fig. 2 C). Alae on either side of the base of the microphyll up to 4.5 cm tall, pale brown. Ligule narrowly elongate to triangular. Labium spathulate. Velum covering < 20% of sporangium. Sporangium basal, oval, 4–6 mm long and 1–1.5 mm wide, lightly brown-streaked. Megaspore white, 616–946 μm in diameter, $\bar{x} = 753$ μm; cristate to rugate proximally, the ornamentation sparse to dense; cristate to reticulate distally, with tall, thick-walled muri of even height; proximal surface with bold tri-radiate ridges; equatorial ridge with an obscure to slightly papillate girdle below. Microspores light gray in mass, 33–40 μm long, $\bar{x} = 36$ μm; laevigate. Chromosome number $2n = 88$.

PARATYPES.—U.S.A. Tennessee: **Monroe Co.**, Little Tennessee River: Jones Ferry, *B.E. Wofford et al. 78–133* (TENN); Tomatlo Ford, *B.E. Wofford and W. M. Dennis, 78–134* (TENN); southwest end of Davis Island, *B.E. Wofford and W. M. Dennis, 78–135* (TENN); gravel bars several miles upstream from hwy 411 bridge, *B. Boom 318* (TENN); upstream side of Davis Island near Mile 15, *W.M.*

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FIG. 1. SEM photomicrographs of Isoëtes *lacustris* and *I. tennesseensis*. A–D. *I. lacustris*. A–C. *Taylor 4902* (MIL): A: proximal view of megaspore; B. distal view of megaspore; C. lateral view of megaspore. D. *Taylor 5010* (MIL): Microspore. E–H. *I. tennesseensis*; *Budke et al. 17* (MIL—holotype): E. proximal view of megaspore; F. distal view of megaspore; G. lateral view of megaspore; H. microspore.

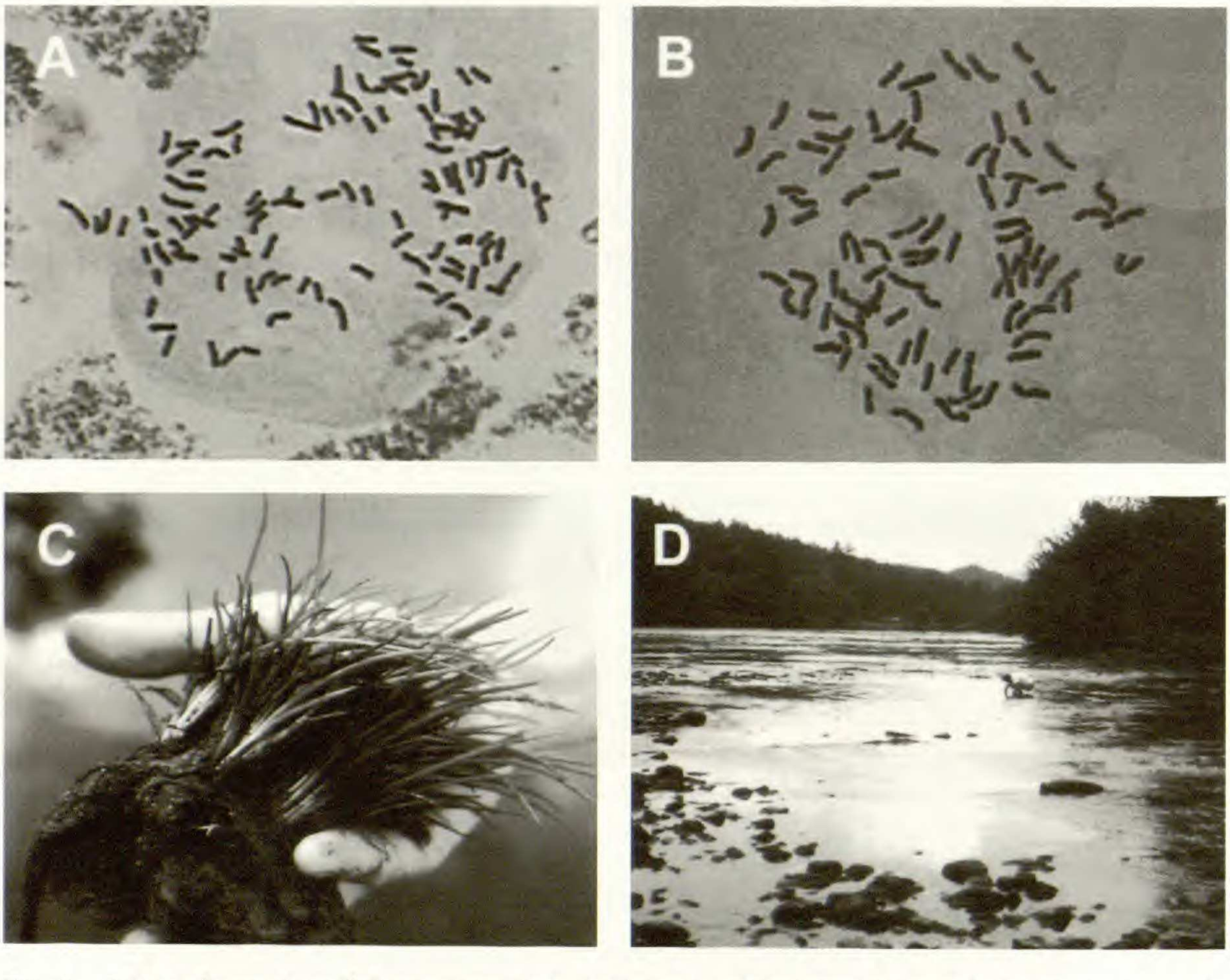

FIG. 2. *Isoëtes lacustris* and *I. tennesseensis*. A. Somatic chromosomes in mitotic root tip squash of *Isoetes lacustris, Jermy 22931* (MIL). B. Somatic chromosomes in mitotic root tip squash of *I. tennesseensis, Taylor 6153* (MIL). C. Plants of *I. tennesseensis*. D. Habitat shot of Hiwassee River, 1.6 mi downstream from Reliance, Tennessee.

Dennis et al. (TENN). **Polk Co.**, Hiwassee River: shallow shoals at intersection of Hwy. 30 and State Road 2518, *B.E. Wofford and A.M. Evans 78–168* (TENN); along Hwy 30 ca. 0.6 mi NW of bridge at Reliance, *W.C. Taylor 5189* (MIL); at Tenn 315 and 30, Reliance and scattered 1.6 mi downstream, *K.D. Heafner et al. 00-042* (MIL, MU); ca. 0.25 mi upstream of crossing of Tellico-Reliance Road, *J. Budke et al. 8* (MIL, MU).

Distribution.—At present, *Isoëtes tennesseensis* is known from southeastern Tennessee, in the Little Tennessee River in Monroe County and the Hiwassee River in Polk County. Specimens of *I. tennesseensis* have not been found in the Little Tennessee River since the construction of a dam and the permanent raising of the water level. However, it is likely that plants could persist in some areas of this river where conditions are suitable.

Isoëtes tenneseensis grows in the cool waters of the Hiwassee River (Fig. 2 D). An upstream dam results in water levels rising and falling on a regular basis. On average the water is two meters deep but can vary across the river. Plants of *I. tennesseensis* are constantly submerged and appear to be obligate

aquatic as evidenced by their lack of stomata. River substrate varies, including cobble, sand, and crevice-ridden shale. Plants were found growing wedged in the sand-filled crevices of the shale or partially buried in sandy cobble.

To date, *Isoëtes tennesseensis* has only been found in a few locations along the Hiwassee River. Searches for the plant farther upstream from the known locations and in other river systems have not revealed other populations. It is unknown whether *I. tennesseensis* still occurs in the Little Tennessee. Further field investigation is necessary. Until more is known about the species' distribution, it is suggested that the known populations be afforded protection.

It does not appear that the population at Passage Creek, Virginia is this new species. Rebecca Bray has counted the chromosomes from these plants and reports that they are $2n = 110$ (Personal Communication). Examination of specimens from this population also reveals that they differ from *I. tennesseensis* in leaf and spore morphology, but are similar to *I. lacustris*. Megaspores range in size from 580–705 μm and fall within the range for *I. lacustris*.

Kott and Britton (1983) found that spore size can be correlated with ploidy level in *Isoëtes*. This does not seem to hold with this species since megaspore size of the octoploid, *I. tennesseensis* ($\bar{x} = 753$ μm) is larger than that for the decaploid, *I. lacustris* ($\bar{x} = 640$ μm). However, this correlation between ploidy level and spore size is reflected in the microspore size where those of *I. tennesseensis* are smaller ($\bar{x} = 36$ μm) in comparison to those of *I. lacustris* ($\bar{x} = 43$ μm).

Isoëtes tennesseensis is the only octoploid quillwort reported for North America and only the third worldwide. The others are *I. pseudojaponica* M. Takamiya, Mitsu. Watan. & K. Ono which occurs in Japan (Takamiya, 1999; Troia, 2001) and *I. andina* Hook. from South America (Taylor *et al.*, 2002).

Preliminary studies of comparisons of nuclear ribosomal ITS nucleotide sequences suggest a possible origin of *I. tennesseensis*. The comparison indicates that *I. tennesseensis* is similar to *I. engelmannii* A. Braun and *I. valida* (Engelm.) Clute and shares several ITS nucleotide sites and indels with each. *Isoëtes engelmannii* and *I. valida*, both diploids ($2n = 22$), and their allotetraploid ($2n = 44$), *I. appalachiana* D. F. Brunton & D. M. Britton (Napier *et al.*, 2002) are sympatric within the area of *I. tennesseensis*. Further comparison of six cloned ITS genomic sequences showed all six were similar to *I. engelmannii*. From these preliminary studies a pedigree is proposed for *I. tennesseensis* that suggests it is the result of the backcrossing of *I. engelmannii* with *I. appalachiana* to form a sterile triploid ($2n = 33$) which doubled its chromosomes to form a fertile hexaploid ($2n = 66$). The result of *I. engelmannii* backcrossing with this hexaploid would produce a sterile *tetraploid* that with the doubling of its chromosomes would produce a fertile octoploid. Further molecular investigations of *I. tennesseensis* may reveal more information about the origin of this new species.

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